Docket No.: 30187/41217
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Martin Kintrup et al.

Application No.: 10/533,618 | Confirmation No.: 1749

Filed: February 6, 2006 | Art Unit: 1645

For: MEANS AND METHODS FOR DIAGNOSING A TREPONEMA INFECTION | Examiner: B. J. Gangle

SECOND DECLARATION UNDER 37 C.F.R. § 1.132

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

I, Martin Kintrup, Ph.D., declare that:

1. I am a co-inventor named on United States Patent Application No. 10/533,618. I am currently employed as Director Sales and Marketing at Viramed Biotech AG, Behringstrasse 11, 82152 Planegg, Germany, assignee of the instant application. I served as Director of Research and Development at Viramed Biotech AG between 2001 and 2004. The Treponema + VDRL ViraBlot® Tests were developed with my direction. I also developed the following test systems for human diagnostics: EBV ViraStripe® (1999), *Bordetella pertussis* ViraBlot® (2000), ENA ViraStripe® (2000), Leber ViraStripe® (2002), Treponema + VDRL ViraBlot® (2002), Bordetella pertussis ViraStripe® (2003). This is the second declaration under 37 C.F.R. § 1.132 that I have provided for this application. My education and experience are summarized in paragraphs 2-4 of my first Rule 1.132 declaration, submitted February 4, 2009.

2. The specification of U.S. Patent Application No. 10/533,618 ("the patent application") describes the successful generation of a test strip comprising both cardiolipin and immobilized *Treponema*-specific antigen. The method used to prepare the test strip is described at pages 10-12 of the specification. VDRL antigen was prepared from a mixture of cardiolipin, lecithin, and cholesterol (page 10, first paragraph). The mass ratios of cardiolipin:lecithin:cholesterol in the VDRL antigen were in the range of (0.1-4.0):(0.1-5.0):(0.1-10.0). Each component was diluted in ethanol, mixed, and the resulting composition was diluted in phosphate-buffered saline prior to application to the nitrocellulose carrier (page 10, first paragraph). The amount of ethanol in the diluted VDRL antigen composition was in the range from 0.8% to 50% (v/v). Four *Treponema* antigens were prepared from *Treponema pallidum*: the 47 kD protein, the 44.5 kD protein, the 17 kD protein, and the 15 kD protein (see paragraph bridging pages 10-11). Four different concentrations of VDRL antigen and each of the four *Treponema* antigens were applied to a nitrocellulose carrier using an automatic dispenser (page 11, first paragraph). Free binding sites were blocked by first incubating the nitrocellulose in a trishydroxymethyl-aminomethane buffer comprising sodium chloride, TWEEN® 20 (3.83 ml/l), thimerosal, and milk powder (40 g/l), then incubating the carrier in a similar buffer comprising 5 g/l milk powder (page 11, second paragraph). The resulting carrier was successfully used to detect both anti-cardiolipin antibodies and antibodies specific for *Treponema*-specific antigen in patients suffering from *Treponema* infection. Test strips prepared using the method described at pages 10-12 of the specification are illustrated in Figures 1, 2, 4, and 5. The test strip in Figure 1 comprised serum and cut-off controls in addition to cardiolipin and *Treponema*-specific antigens.

3. In my declaration of February 4, 2009, I described some of the shortcomings of methods for preparing test strips disclosed in the scientific literature published prior to the filing of the patent application. I also described a failed attempt to generate a nitrocellulose carrier comprising immobilized cardiolipin and immobilized 47 kD *Treponema* antigen employing the same detergent concentration disclosed in the Sambri reference (*Clinical and Diagnostic Laboratory Immunology*, *8*(3), 534-539 (2001)), 0.05%

TWEEN® 20. My comments regarding our previous experiment do not contradict the teachings of the patent application.

4. Several parameters influence antigen-antibody test systems. One of these parameters is the detergent concentration in a buffer, which can greatly affect antigen structure and adherence to a carrier. The experiment described in paragraph 8 of my previous declaration used a buffer similar to the Sambri buffer, and the buffer released VDRL antigen from the test strip. The working method provided in the application, however, used a different buffer than that disclosed in the Sambri reference. For convenience, the chart in Appendix A (provided herewith) summarizes (a) the buffer composition of the Sambri reference, which mirrors the blocking and serum incubation/wash conditions used in the experiment described in paragraph 8 of my previous Rule 132 declaration, and (b) the buffer composition of the working example in the application. The serum incubation/wash buffers of the Sambri reference comprised only sodium phosphate, sodium chloride, and 0.05% TWEEN® 20, and did not contain milk powder. In contrast, the working example of the patent application used different conditions, i.e., TRIS-buffer (3.25 g trishydroxymethylaminomethane and 7.5 g NaCl per liter (i.e., 0.75% NaCl)), TWEEN® 20 (3.83 ml per liter), and milk powder (5.0 g per liter) (page 11, fourth paragraph). TWEEN® 20 has different effects on immobilized antigens in the context of the different buffer compositions, and the different experimental conditions disclosed in the patent application allowed us to use a higher concentration of the detergent. The conditions disclosed in the cited art solubilized lipid antigens, as illustrated by the depiction of test strips in Appendix A of my first Rule 1.132 declaration. In contrast, the working example of the application generated a carrier comprising immobilized protein and lipid antigens that successfully detected anti-*Treponema* antibodies, as illustrated by Figures 2, 4, and 5 of the application. It was surprising that cardiolipin reactivity could be maintained at a level required for a diagnostic test while maintaining sensitive and selective reactivity of *Treponema* protein antigens to anti-*Treponema* antibodies on the same carrier.

5. In my declaration of February 4, 2009, I also commented on the method of adhering cardiolipin to a substrate disclosed in Pedersen et al., *J. Clin. Microbiol.*,

25(9): 1711-1716 (1987), which is not suitable for use with protein antigens. The Pedersen reference teaches that polyvinyl chloride microtiter plates were *coated* with an ethanol solution containing VDRL antigen, and the ethanol was evaporated overnight (page 1712, column 1, paragraph 3). Coating a carrier with ethanol would disrupt protein antigens, rendering a carrier comprising both cardiolipin and a *Treponema*-specific antigen unable to detect anti-*Treponema* antibodies. In addition, high concentrations of ethanol can destroy nitrocellulose.

6. The working example in the patent application solves the problems caused by ethanol when generating nitrocellulose carriers comprising both protein and lipid antigens. First, the VDRL composition, which comprised ethanol, was diluted in phosphate-buffered saline prior to application to the nitrocellulose carrier (page 10, second paragraph). The amount of ethanol in the diluted VDRL antigen composition was in the range from 0.8% to 50% (v/v), such that the carrier sustained minimal damage. Second, the patent application teaches dripping the VDRL solution onto the test strip, unlike the Pedersen method which requires coating the carrier with ethanol (page 11, first paragraph). Dripping an ethanolic solution containing lipid antigen at discrete location(s)/spots(s) on a carrier ensures that protein antigens immobilized at other discrete location(s)/spot(s) on the same carrier are not disrupted. (See, e.g., Figures 2, 4, and 5 of the patent application.) Dripping the solution onto the carrier also minimizes any damage to the nitrocellulose carrier. The carrier produced using the techniques described at pages 10-12 of the patent application contained immobilized cardiolipin and immobilized Treponema-specific antigen, and was successfully used to detect anti-*Treponema* antibodies in infected patients.

7. I declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

July 31st 2009
Date

Martin Kintrup, Ph.D.

APPENDIX A

Summary of Buffer Compositions for Blocking and Serum Incubation of Western Blots described in Sambri et al., 2009 and U.S. Patent Application No. 10/533,618

Step	Sambri et. al, 2001 whole cell sonicate (wcl)	Working Example of U.S. Patent Appln. No. 10/533,618
Blocking	not shown	(p. 4, paragraph [0059]) 3.25 g/l trishydroxymethylaminomethane (0.33%) 7.51 g/l sodium chloride (0.75 %) 3.83 g/l TWEEN20® (0.38%) 40.0 g/l milk powder (4.0%, step 1) 5.0 g/l milk powder (0.5%, step 2) pH = 7.5, temp.: 37°C (98.6 °F) 0.02 g/l Thimerosal
Serum incubation / Wash	(p. 535, right col., line 6) serum dilution: 1:100 phosphate buffered saline (PBS) sodium phosphate 9.00 g/l sodium chloride (0.9%) 0.5 g/l TWEEN20® (0.05%) (no milk powder) *= blocking and serum incubation/wash conditions used in experiment described in paragraph 8 of previous Rule 132 declaration*	(p. 4, paragraph [0062]) serum dilution: 1:75 (20 µl / 1.5 ml) 3.25 g/l trishydroxymethylaminomethane 7.51 g/l sodium chloride 3.83 g/l TWEEN20® (0.38%) 5.0 g/l milk powder (0.5%) pH = 7.5, room temp. 0.02 g/l Thimerosal